HARVEST AND ISOLATE SKELETAL MUSCLE SATELLITE CELLS FROM RATS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Literature Review

Skeletal muscles have the ability to rebuild themselves after injury and/or grow after atrophy, and this adaptability is due, in part, to satellite cells. Satellite cells, also known as adult stem cells, are found between the sarcolemma and basement membrane on a muscle fiber. These cells are responsible for creating new muscle fibers and/or combining with existing myofibers to facilitate growth and repair. After an injury, satellites cells begin to proliferate to produce myoblasts to facilitate the regeneration of muscles. Understanding the contributions of satellite cells as contributors to the adaptability of skeletal muscle may lead to important medical contributions for a number of degenerative diseases like muscular dystrophy. The first aim of the proposed studies for this Undergraduate Honors Thesis was to harvest and isolate satellite cells from intact muscle of rats, and subsequently grow these cells into viable myotubes in culture. The second aim of these studies was to characterize the cultured myotubes and compare anabolic properties to existing intact animals. Outcomes for these studies will be to compare rates of protein synthesis of skeletal muscle, as well as signaling outcomes between cultured or intact muscle in an effort to understand morphological/metabolic differences between these conditions. The central hypothesis for the proposed study is that some of the phenotypic

expression of skeletal muscle (fiber type, metabolic profiles, anabolic profiles) will persist in cell culture. If the harvested cells maintain similar characteristics to the intact tissue we can then use these methodologies to better understand mechanisms of muscle disease states in culture, and potentially, translate our findings/interventions to intact living organisms. Ultimately, if successful, we could then use these methodologies to isolate satellite cells from biopsied human tissue, and subsequently grow these cells into differentiated myotubes from individuals with specific muscle abnormalities to systematically design therapies/interventions with an on-going human cell line model, particularly if the abnormality is sustained from the host to the cell cultured environments. This would allow scientists to harvest and maintain human cells without the constant need to obtain muscle biopsies from the host.

Thesis Statement

To establish methodologies that allow for the growth and differentiation of satellite cells harvested from intact muscle tissue under cultured conditions.

To determine if cultured cells adapt/respond similarly to cells from intact muscles.

Theoretical Framework

The isolation of satellite cells is a very complex and difficult protocol. If successful, we can use these methods to isolate human satellite cells and have the cultured myotubes differentiate into functionally contracting myotubes. This could, indefinitely, allow growth of cell lines of various diseased tissues.

Project Description

In the Muscle Biology Lab with Dr. Fluckey, I had isolated and harvested satellite cells from muscle tissue of rats. The rats were dissected to obtain one of the following muscles: tibialis anterior, soleus, or gastrocnemius. The obtained muscle tissue was cleaned with alcohol

and placed into a petri dish for further rinsing. The muscle tissue was then transferred into a clean plate that contained phosphate-buffered saline (PBS) with antibiotics (AB) and antimycotics (AM). After the initial rinse, the tissue was placed into a weigh boat plate which also contained PBS with AB/AM. The muscle was then cleaned by removing visible fat and connective tissue. The muscle tissue was then placed in a plate containing collection media. The collection media consist of Dulbecco's Modified Eagle Medium (DMEM) and penicillin/streptomycin/amphotericin B. While the muscle tissue sits in the plate for a few minutes, it allowed me to see the connective tissue better. I then continued to remove any visible connective tissue and then placed it into a glass 100mm plate with collection media. The muscle tissue was then minced using sterile forceps against a #10 scalpel. The minced muscle was then transferred to a 15mL conical and rinsed with 5mL of collection media. The muscle tissue was then placed in the centrifuge at 800-1000xg for 2 minutes. The media was then aspirated after the centrifuge step and the tissue was resuspended in 10mL of collection media. The solution was decanted into a clean 100mm dish and rinsed with 8mL of collection media which was then added to another clean dish. 2mL of 1% pronase was added to the dish along with a sterile bar. The dish was then placed in a CO_2 incubator and the stir plate was turned on at level 5 or a slow spin without splashing. The dish was left in the incubator for one hour. After one hour, the dish was transferred to a 50mL conical and centrifuged for 3 minutes at 800-1000xgs. The muscle tissue was resuspended with 10mL of DMEM with 10% fetal bovine serum (FBS) and penicillin/streptomycin. Using a 10mL pipet, the muscle digest was triturated 15-20 times to loosen cells if clumps formed. The pieces were allowed to settle and by using a sterile 6" Pastuer pipet it was transferred to a clean 50mL conical. 10mL of post digestion media was then added to the tissue pieces which contained 90% DMEM, 10% FBS, and

penicillin/streptomycin/amphotericin B. This step was repeated for a total of two rounds. The supernatant was then passed through a Steriflip 100µm vacuum filter which was washed with 5mL of post digestion media. The cell suspension was then passed through a 70µm cell strainer and washed with 5mL of post digestion media and finally with a 40µm filter. The muscle tissue solution was placed in the centrifuge for 5 minutes at 1000xg. The pellet was resuspended in 10mL of post digestion media and plated on a 100mm TC dish which was then placed in the incubator for 45 to 60 minutes. After, the media and suspended cells were transferred to a 15mL conical and spun at 1000xg for 5 minutes. While spinning, a supplement of 10mL of growth media which contained: 80% Hams-F10, 20% FBS, penicillin/streptomycin/amphotericin B, and 5mg/mL basic fibroblast growth factors (bFGF) was created for growth media. From the cell pellet, the media was aspirated and placed in the growth media +bFGF. This was then plated on a collagen coated plate and returned to the incubator for 3 days. After 3 days, the plated cells were fed with growth media that was supplemented with bFGF every other day from here on. The isolation of satellite cells has shown high levels of difficulty in collecting pure satellite cells. There is a high risk in growth of fibroblasts during culture and potential contaminations. It is recommended that the mice are 6 to 12 weeks of age because of the high levels of myoblasts.

After 3 days of incubation, the plates were analyzed for growth of muscle fibers. The results showed no growth in muscle tissue and only contained cell debris. When the muscle cells were collected they were then frozen and stored for 2 weeks until cultured. Unfortunately, when the tissues were thawed, it was apparent that the cells did not thrive after the freezing process, likely because nutrients were depleted during their time in the freezer which may have potentially caused the cells to not be able to differentiate into myofibers in culture. As a result of the failed myotube transition, the next iteration of the experiments will be to harvest muscles and

immediately begin the protocols for isolation without freezing. The muscle tissues will be fresh and supplemented with proper nutrients for isolation and harvest. The methodologies will also be performed exclusively in a biosafety cabinet for a more aseptic technique to prevent any contamination of the cells. The next set of samples will be performed with these new proposed methodologies to increase the success of harvesting, isolating and culturing satellite cells.

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INTRODUCTION

Wedged in between the basement membrane and plasma membrane are satellite cells. These cells are known as adult myoblasts which have been proven to contribute to the growth and development of muscle. After an injury, satellite cells are activated to contribute to muscle repair. Experiments have shown that the isolated satellite cells can be grown in *ex vivo* (1). If successful, the methodologies can be used to study potential myoblast transplantation as a treatment for muscular dystrophy. With the harvested cell cultures, we can then apply different muscular diseases and further our knowledge on the mechanism of the diseased cells. Duchenne and Becker muscular dystrophies (MD) are genetic diseases that cause progressive weakness and degeneration of skeletal muscles. Duchenne MD is the most common childhood form of MD, which affects approximately 1 in every 3,500 to 6,000 male births each year in the United States (2). In 2007, muscular dystrophy has been reported in 349 of 2.37 million males aged 5-24 years old (3). As a genetic disorder, there are no specific treatments that can prevent or cure any form of muscular dystrophy. With successful methodologies, scientists may be able to further study the mechanism of the harvested cells and apply them to medicine against muscular diseases. Studies have shown that the function of satellites cells depend on the dystrophic environment of the tissue (4). Muscle tissue with muscular dystrophy, has shown the lack of dystrophin which leads to satellite cells to not functioning properly (4). With further studies on satellite cells, the methodologies presented would allow the expression of phenotypic characteristics of cultured cells and, essentially, allow the growth of differentiated myotubes to study different muscular diseases and their effects on satellite cells and myotube growth.

CHAPTER I

PHENOTYPIC EXPRESSION OF SATELLITE CELLS

During stages of myogenesis, myotubes can differentiate into particular myofibers. The muscle fibers can become slow-twitch fibers, or type I, which are muscles that are used for posture, routine locomotion and during high levels of aerobic exercise (5). Fast-twitch fibers, or type II, are used for short powerful burst of movement (5), mostly due to their inability to maintain prolonged activity. During myogenesis, muscle fibers are created based on the environment of the growing embryo and postnatal development. Satellite cells are formed during embryonic development and are stored in a quiescent state in the adult muscles until restoration of muscle and/or myonuclei are needed (6). In this research study, muscle samples from different anatomical regions of the mouse will have their satellite cells harvested, isolated and cultured into myotubes in order to determine if specific phenotypes of the host muscle will be maintained in culture. A study from Drexel University School of Medicine suggested that the mouse satellite cells isolated from their hosts have differentiated consistently, irrespective to their anatomical location, fiber type, and embryonic origin of the donor muscle (7). While those results can conclude that the phenotypic expression of that satellite cell delivers an undeviating cell source which can further be studied for transplantation research (7), it may also offer fewer opportunities to assess disease states, such as those involving metabolic abnormalities, in culture.

CHAPTER II

MICROENVIRONMENT OF SATELLITE CELLS

Recent developments have brought extensive prominence on the microenvironment or "stem cell niche" that has proven to be very specific and significant to the mechanism of satellite cells and myogenic function (8). Muscle stem cells are known to repair damaged muscle tissue upon injury but also undergo self-renewal. This self-renewal characteristic allows the satellite cells to consistently have newly quiescent satellite cells. Researchers have not fully understood the molecular mechanisms for satellite cell self-renewal but the stem cells generate progeny that replenishes the pool of quiescent satellite cells. A study on division of satellite cells in single myofibers demonstrated that the stem cells indeed undergo asymmetrical and symmetrical division (9). Stem cells in the asymmetric division model results from one stem cell producing one differentiated cell and one stem cell. The result of the symmetric division is a stem cell producing two differentiated cells or two stem cells. The mechanism of asymmetric to symmetric division greatly depends on the absolute position of the daughter cells to the myofiber. This discovery shows strong indications that the satellite cell self-renewal is controlled by the structure and signaling present in their niche (9). The ability to identify the many variables in the mechanism of satellite cell self-renewal would, theoretically, allow scientists to manipulate these variables to potentially control the differentiation of the satellite cells. This would greatly impact the research on muscle regeneration and medical research on muscular diseases. With the collection of research on satellite cell differentiation, it can be concluded that the function of the satellite cells greatly depends on their surroundings. Numerous research studies have found that satellite cell progeny cultured on regular collagen-coated plastics dishes and influenced with cell-

derived primary myoblasts are molecularly and functionally different from the satellite cells freshly isolated from muscles (10, 11, 12). Therefore, advancement of this research may allow for muscle studies, in culture, to better mimic the intact physiological state, which may have important implications for our understanding of specific diseases that have remained elusive using current, in vivo, approaches.

CHAPTER III

SATELLITE CELL TRANSPLANTATION

Human tissue engineering is an ongoing research endeavor that could be critical to modern day medicine. The ability to use cultured myoblasts to transplant to a human host could lead to new therapies for multiple skeletal muscle diseases. Generally, experiments involving human muscle stem cell transplantation yield low efficiency and inefficient satellite cell functions for self-renewal or expansion after injury (13). There is evidence from a study conducted at the University of California that reported successful transplantation of adult human muscle stem cells from diverse muscles by using direct transplantation of niche-protected satellite cells on fibers and transplantation of flow-cytometry-enriched satellite cells (14). These results illustrate that the methodologies, similar to those practiced in the present study, can form key contributions to successful transplantation of satellite cells. By performing successful isolation of satellite cells followed by adequate methodologies for differentiation, we can then expand upon those methodologies for transplantation of functional satellite cells to different hosts resulting in a medical breakthrough for muscle regeneration. One of the objectives of this study is to develop methods that would allow these isolated satellite cells to maintain their undifferentiated state in culture which would, ultimately, increase the proficiency of transplantation therapies. Muscle stem cell research is an ongoing study that could open doors to medical discoveries in regards to therapies for muscular diseases.

CONCLUSION

Unfortunately, the results of the experiment concluded that the previous techniques did not successfully isolate and culture satellite cells from harvested rat tissue. These methodologies have been modified, and subsequent, on-going attempts to culture harvested satellite cells are being performed in an attempt to have successful outcomes. With a more aseptic protocol and fresh muscle tissue, the methodologies will hopefully yield growing myotubes from the isolated tissue samples in the very near future. The previous methodologies were performed with dissected rat tissues and the next trial will be conducted with tissues collected from mice, where the lab has extensive experience in culturing undifferentiated mouse myoblasts.

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